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INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 85336	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. PCT/AU99/00563	International filing date (day/month/year) 12 July 1999	Priority Date (day/month/year) 10 July 1998
International Patent Classification (IPC) or national classification and IPC Int. Cl. ⁷ C07K 14/38, A21D 2/38, A23L 1/10		
Applicant COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 3 sheets, including this cover sheet.
- ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 6 sheet(s).

3. This report contains indications relating to the following items:

- | | | |
|------|-------------------------------------|---|
| I | <input checked="" type="checkbox"/> | Basis of the report |
| II | <input type="checkbox"/> | Priority |
| III | <input type="checkbox"/> | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | <input type="checkbox"/> | Lack of unity of invention |
| V | <input checked="" type="checkbox"/> | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | <input type="checkbox"/> | Certain documents cited |
| VII | <input type="checkbox"/> | Certain defects in the international application |
| VIII | <input type="checkbox"/> | Certain observations on the international application |

Date of submission of the demand 10 February 2000	Date of completion of the report 31 July 2000
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer GAVIN THOMPSON Telephone No. (02) 6283 2240

INTERNATIONAL PR

M. EXAMINATION REPORT

International application No.

PCT/AU99/00563

I. Basis of the report

1. With regard to the elements of the international application:*
- ☐ the international application as originally filed.
- ☒ the description, pages 1, 2, 4 to 29 as originally filed,
pages 3, 30 received on 1 June 2000 with the letter of 31 May 2000
pages 46 received on 25 July 2000 with the letter of 25 July 2000
- ☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages 43 to 45 received on 1 June 2000 with the letter of 31 May 2000
- ☒ the drawings, pages 1/17 to 17/17, as originally filed,
pages , filed with the demand,
pages received on with the letter of
- ☒ the sequence listing part of the description:
pages 31 to 42 as originally filed
pages , filed with the demand
pages , received on with the letter of
2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:
- ☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).
3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, was on the basis of the sequence listing:
- ☐ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished
4. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
- ☐ the claims, Nos.
- ☐ the drawings, sheets/fig.
5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

INTERNATIONAL PRIORITY EXAMINATION REPORT

International application No.

PCT/AU99/00563

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**1. Statement**

Novelty (N)	Claims 1 to 27	YES
	Claims	NO
Inventive step (IS)	Claims 1 to 27	YES
	Claims	NO
Industrial applicability (IA)	Claims 1 to 27	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

The instant claims are novel and inventive over the prior art as the insertion of exogenous amino acid domains into glutenin and seed-storage protein which allow them to be mixed with gluten and also to bind to macromolecular ligands is not disclosed in it. The gluten mixed with these proteins is used in food applications. The prior art discloses the insertion of endogenous amino acid domains into glutenin to improve the viscoelasticity of their bread doughs.



To alter protein-protein, protein-lipid and protein-starch interactions within the gluten matrix, the present inventors have developed a system which enables the incorporation of new surface active molecules or parts of molecules into the gluten matrix.

5 Disclosure of Invention

 In a first aspect, the present invention consists in a method of producing a modified glutenin or seed-storage protein, the method comprising adding to the protein a domain which confers to the modified protein the ability to incorporate into gluten or bind a ligand or other
10 macromolecule.

 In one preferred embodiment, the modified glutenin or seed-storage protein contains one or more amino acid residues added to its amino acid sequence. More preferably, the one or more amino acid residues are one or more cysteine residues. Preferably, the one or more cysteine residues are
15 incorporated at one or both ends of the amino acid sequence of the protein. The addition of the one or more cysteines allows the modified proteins to be more easily incorporated into gluten in use. The modifications to the glutenin or seed-storage proteins produced according to the present invention allow the incorporation of that protein into the gluten network for food or
20 industrial use.

 The present inventors have found that incorporating exogenous amino acid sequences (domains) from proteins other than glutenins into glutenin or seed-storage proteins modifies the general properties of gluten when the proteins are used in a range of food applications.

25 Figure 15 provides a schematic of the scheme for identifying transgenes in transformed wheat plants by polymerase chain reaction (PCR). A primer pair straddling the interface between the gene and its promoter (from the gene for the high molecular weight glutenin Bx17 for example) ensures that no false positives are detected arising from the high homology
30 between C hordein and gliadin genes.

 In a further preferred embodiment, the domain is a binding domain that confers to the modified protein the ability to bind a ligand or other macromolecule.

 The binding domain can be any domain that will bind ligands that may
35 be useful in food preparation or in food compositions. In a preferred form, the binding domain is a ligand capable of binding lipids or starches. The

Le Gal-Coeffet, M-F., Jacks, A.J., Sorimachi, K., Williamson, M.P., Williamson, G. and Archer, D.B. 1995. Expression in *Aspergillus Niger* of the starch-binding domain of glucoamylase. *Eur. J. Biochem.* 233:561-567

MacRitchie, F. 1992. Physicochemical properties of wheat proteins in relation to functionality. *Adv. Food Nutr. Research* 36:1-87

Morrison, W.R. 1989. Recent progress on the chemistry and functionality of flour lipids. Pages 131-149 in: *Wheat end-use properties: Wheat and flour characterisation for specific end-uses*. H. Salovaara, Ed. University of Helsinki, Lahti.

Tamas, L., Bekes, F., Greenfield, J., Tatham, A.S., Gras, P.W., Shewry, P.R. and Appels, R. 1998. Heterologous expression and dough mixing studies of wild-type and mutant C hordeins. *Journal of Cer. Science* 27:15-22

Weeks, J.T., Anderson, O.D. and Blechl, A.E. 1993. Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*). *Plant Physiology* 102:1077-1084

Witzens, B., Brettell, R.I.S., Murray, F.R., McElroy, D., Li, Z., Dennis, E.S. 1998. Comparison of three selectable marker genes for transformation of wheat by microprojectile bombardment.

CLAIMS:

1. A method of producing a modified glutenin or seed-storage protein, the method comprising adding to the protein a domain which confers to the modified protein the ability to incorporate into gluten or bind a ligand or other macromolecule.
2. The method according to claim 1 wherein the domain comprises one or more amino acid residues.
3. The method according to claim 2 wherein the one or more amino acid residues comprise one or more cysteine residues.
4. The method according to claim 3 wherein the one or more cysteine residues are incorporated at one or both ends of the amino acid sequence of the protein.
5. The method according to claim 1 wherein the domain is a binding domain that confers to the modified protein the ability to bind a ligand or other macromolecule.
6. The method according to claim 5 wherein the modified protein further contains one or more amino acid residues which confer to the protein the ability to incorporate into gluten.
7. The method according to claim 5 or 6 wherein the binding domain is capable of binding lipids or starches.
8. The method according to claim 7 wherein the lipid-binding domain is derived from barley oleosin gene or the lipid-binding regions of wheat CM16 protein.
9. The method according to claim 7 wherein the starch-binding domain is derived from glucoamylase from *Aspergillus niger*.
10. The method according to any one of claims 1 to 9 wherein the glutenin or seed-storage protein is selected from the group consisting of low molecular weight glutenins, high molecular weight glutenins, gliadins, puroindolines, grain softness proteins, friabilins, and Chloroform/Methanol-soluble proteins.
11. The method according to claim 10 wherein the glutenin or seed-storage protein is C hordein from barley.
12. A modified glutenin or seed-storage protein having a domain inserted therein which confers to the protein the ability to incorporate into gluten or bind a ligand or other macromolecule.
13. A modified glutenin or seed-storage protein produced by the method according to any one of claims 1 to 11.

14. A modified glutenin or seed-storage protein selected from the group consisting of ANG/SBD/Cys7Cys236, ANG/OHBD/Cys7Cys236, and ANG/CM16/Cys7Cys236, as hereinbefore defined.

15. An isolated nucleic acid molecule encoding a modified glutenin or seed-storage protein according to any one of claims 11 to 14.

16. A cell containing an isolated nucleic acid molecule according to claim 15 such that on expression of the nucleic acid molecule, the cell produces the modified glutenin or seed-storage protein.

17. The cell according to claim 16 selected from bacteria, yeast, plant, insect or mammal.

18. The bacterial cell according to claim 17 being *Escherichia coli*.

19. The yeast cell according to claim 17 being *Pichia* sp. or *Saccharomyces cerevisiae*.

20. The plant cell according to claim 17 being a recombinant wheat cell.

21. Use of a modified glutenin or seed-storage protein according to any one of claims 11 to 14 in the preparation of a food product.

22. The use according to claim 21 wherein the food product is selected from the group consisting of leavened or unleavened breads, pasta, noodles, breakfast cereals, snack foods, cakes, pastries, and foods containing flour-based sauces.

23. Use of a modified glutenin or seed-storage protein according to any one of claims 11 to 14 in the preparation of a non-food product.

24. The use according to claim 23 wherein the non-food product is selected from the group consisting of films, coatings, adhesives, building materials, and packaging materials.

25. Use of a grain or part of a grain containing a modified glutenin or seed-storage protein according to any one of claims 11 to 14 in the preparation of a food product.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00563

A. CLASSIFICATION OF SUBJECT MATTER												
Int Cl ⁶ : C07K 14/38, A21D 2/38, A23L 1/10												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols)												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) 1) File CA, WPIDS: (glutenin or gliadin or puroindole or friabilin) and (modif?) and (cysteine or cys or nucleic(w)acid or gene or transgenic); 2) Gen Bank, Swiss Prot, EMBL, PIR: SEQ ID NO 19												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
X	WO 98/08607 (The United States of America represented by the Secretary of Agriculture) 5 March 1998 Pages 3-6 and claims	1-25										
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex												
<p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 18 August 1999		Date of mailing of the international search report 23 AUG 1999										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929		Authorized officer CHRISTINE BREMERS Telephone No.: (02) 6283 2313										

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International application No.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Plant Physiology Vol 152 No 6 (1998) page 703-707 Blechl, Ann E. et al "Engineering Changes in Wheat Flour by Genetic Engineering" Page 703 column 2 last paragraph-page 704 column 1 fifth paragraph and summary	1-25
X	Journal of Cereal Science Vol 25 No 1 (1997) pages 1-8 D'Ovidio, R. "Construction of Novel Wheat High-M _r Glutenin Subunit Gene Variability: Modification of the Repetitive Domain and Expression in E.coli" Abstract, page 3 column 1, figure 1	1-25
X	Proceedings-International Wheat Quality Conference (1997) pages 205-211, Blechl, Ann E et al "Applications of Molecular Biology to Understanding and Improving Wheat Quality" Editors: Steele, James L et al Abstract	1-25
X	The Journal of Biological Chemistry Vol 272, No 24 (1997) pages 15488-15495 Shimoni, Yuval et al "A Recombinant Protein of Two High Molecular Weight Glutenins Alters Gluten Polymer Formation in Transgenic Wheat" Abstract, page 15495 column 1 second paragraph	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU 99/00563

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member	
WO	98/08607	AU	41747/97		
END OF ANNEX					